Claims

1. A method for purifying recombinant human FSH or an FSH variant comprising the steps of subjecting FSH to:
   (1) ion exchange chromatography;
   (2) immobilised metal ion chromatography; and
   (3) hydrophobic interaction chromatography (HIC).

2. The method of claim 1, wherein the ion exchange chromatography is carried out with a strong anion exchange resin.

3. The method of claim 2, wherein the anion exchange resin is Q Sepharose FF, or a resin having similar properties.

4. The method of any one of claims 1 to 3, wherein the ion exchange chromatography is carried out using borate buffer as eluent.

5. The method of claim 4, wherein the borate buffer is at a pH of at or about 8.5.

6. The method of any one of claims 1 to 5, wherein the immobilised metal ion chromatography is carried out with a resin having tridentate chelating groups.

7. The method of claim 6, wherein the chelating groups are iminodiacetic acid.

8. The method of any one of claims 1 to 7, wherein the immobilised metal ion chromatography is carried out with chelating Sepharose FF, or a resin having similar properties.

9. The method of any one of claims 1 to 8, wherein the immobilised metal ion chromatography is carried out with a metal ion selected from Cu^{2+}, Zn^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+} and Co^{2+}.

10. The method of any one of claims 1 to 8, wherein the immobilised metal ion chromatography is carried out with Cu^{2+}.

11. The method of any one of claims 1 to 10, wherein the immobilised metal ion chromatography is carried out using ammonium acetate as eluent.

12. The method of claim 11, wherein the ammonium acetate buffer has a pH of at or about 9.

13. The method of any one of claims 1 to 12, wherein the hydrophobic interaction chromatography (HIC) is carried out using Phenyl Sepharose FF HS, or a resin having similar characteristics.

14. The method of any one of claims 1 to 13, wherein the hydrophobic interaction chromatography is carried out using ammonium acetate (50 mM) /ammonium sulphate (0.25 M) as eluent.

15. The method of any one of claims 1 to 14, comprising a second step of ion exchange chromatography (2a), carried out after the step of immobilised metal ion chromatography, and before the step of hydrophobic interaction chromatography (HIC).

16. The method of claim 15, wherein the second step of ion exchange chromatography is carried out using a weak anion exchange resin.

17. The method of claim 16, wherein the weak anion exchange resin is DEAE Sepharose FF resin, or a resin having similar properties.

18. The method of any one of claims 1 to 17, further comprising a step of reverse phase chromatography (4), carried out after the step of hydrophobic interaction chromatography (HIC).

19. The method of claim 18, wherein the reverse phase chromatography is carried out using Source 30 RPC as resin, or a resin having similar characteristics.

20. The method of claim 19, wherein the reverse phase chromatography is carried out using ammonium acetate (50 mM, pH at or about 7.6) with 20% (v/v) 2-propanol.

21. The method of claim 18, 19 or 20, comprising a step of ultrafiltration (5), carried out after the step of reverse phase chromatography.

22. A method for purifying human recombinant FSH comprising the steps of subjecting FSH to:
(0) ultrafiltration;
(1) anion exchange chromatography on Q Sepharose FF with at or about 50 mM borate, at or about 0.13 M NaCl, pH at or about 8.5 as eluent;
(2) subjecting the eluate of step (1) to a step of immobilised metal ion affinity chromatography on chelating Sepharose ff, with Cu^{++} as metal ion, and at or about 0.75 M ammonium acetate pH at or about 9 as eluent;
(2a) subjecting the eluate of step (2) to a step of anion exchange chromatography on DEAE Sepharose FF, with at or about 0.11 M Ammonium acetate, pH at or about 8.5 as eluent;
(3) subjecting the eluate of step (2a) to a step of hydrophobic interaction chromatography on Phenyl Sepharose FF HS with at or about 50 mM ammonium acetate, at or about 0.25 M ammonium sulphate, pH at or about 8.25 as eluent;
(4) subjecting the eluate of step (3) to a step of reverse phase chromatography on Source 30 RPC, with at or about 50 mM ammonium acetate, pH at or about 7.6, with at or about 20% of 2-propanol (v/v);
(5) subjecting the eluate of step (4) to a step of ultrafiltration; and
(6) subjecting the retentate of step (5) to a step of nanofiltration.

23. A purified recombinant human FSH or FSH variant, obtained by the process of any one of claims 1 to 22.

24. A pharmaceutical composition comprising human FSH or FSH variant according to claim 23.